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Marek Černoch

Role RecQ helikáz v údržbě genomové stability během mitózy

Role of RecQ helicases in maintenance of genomic stability during mitosis

Bakalářská práce

Školitel: RNDr. Pavel Janščák CSc.

Praha, 2012

Declaration

I declare that I wrote this work myself and that I stated all information sources and literature I used. This work or its significant part was not submitted in order to obtain another or the same academic title.

In Prague, 23.8.2012

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V Praze, 23.8.2012

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Marek Černoch

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Contents:

Abstract

Helicases are proteins capable of unwinding nucleic acids, their malfunction can be dangerous for genome stability of the cell. Five RecQ-family helicases identified in human cells participate in many cellular events during the whole cell cycle, including mitosis, and therefore are very important for correct functioning. The mutations in RecQ helicases can cause them to malfunction and seriously damage various cell processes, for example DNA replication, DNA damage control or sister chromatids separation. The mutations can also lead to dangerous syndromes, with the hallmark symptom of increased risk of cancer.

Key words: RECQ helicase, mitosis, genome stability, cell cycle

Abstrakt

Helikázy jsou proteiny schopné rozplétání nukleových kyselin, jejich dysfunkce může být nebezpečná pro genomovou stabilitu buňky. Pět helikáz rodiny RecQ identifikovaných v lidských buňkách se podílí na mnoha buněčných pochodech během celého buněčného cyklu včetně mitózy a jsou proto důležité pro jejich správnou funkci. Mutace v RecQ helikázách může způsobit jejich dysfunkci a vážně narušit různé buněčné procesy, například replikaci DNA, kontrolu poškození DNA nebo rozdělení sesterských chromatid. Mutace mohou také vést k nebezpečným syndromům, jejichž typickým příznakem je zvýšené riziko vzniku rakoviny.

Klíčová slova: RecQ helikáza, mitóza, genomová stabilita, buněčný cyklus

List of abbreviations

APC	Anaphase-promoting complex
ATP	Adenosine triphosphate
BGS	Baller-Gerold Syndrome
BS	Bloom Syndrome
CDK	Cyclin-dependent kinase
DNA	Deoxyribonucleic acid
ds	Double-strand
ER	Endoplasmic reticulum
NTP	Nucleoside triphosphate
PARP-1	Poly(ADP-ribose)polymerase-1
PICH	Plk1-interacting checkpoint helicase
RNA	Ribonucleic acid
RTS	Rothmund-Thomson Syndrome
SF	Superfamily
ss	Single-strand
TopoIIIα	Topoisomerase IIIα
TopoIIα	Topoisomerase IIα
UV	Ultraviolet
WS	Werner Syndrome

1. Introduction

One of the most common causes of death in the modern population is cancer, which we still have difficulty dealing with. One of the main reasons is the fact that we still do not have a clear picture about what causes it. The cancer can be triggered by many factors ranging from carcinogenic substances to genetic anomalies. In cells, a large number of proteins are capable of interacting with DNA, possibly altering it or modifying the outcome of DNA metabolism. One type of proteins capable of doing so is the helicase. Several groups, named „families" and „superfamilies", of helicases have been identified in human cells. In this work, I will focus on the RecQ family, whose members, if mutated, are known for participating in cancer development. Their functions and their mechanisms are still not fully understood and are the objects of intense research. This work will try to explore and summarize current knowledge about the RecQ helicase family and their effect on cell cycle and especially mitosis.

2. Helicases

For many cell actions the access to single strand of nucleic acid, be it DNA or RNA, is needed and the proteins that are capable of unwinding double-stranded (ds) nucleic acids are called helicases. They are known to participate in almost every aspect of DNA/RNA metabolism, such as DNA replication. (Tuteja & Tuteja, 2004) In order to accomplish this, they require nucleoside triphosphate for hydrolysis, which is used as an energy source. All helicases contain in their sequence Walker A and Walker B sites that are responsible for binding resp. hydrolysing NTPs.

Presently, helicases were found in every living organism, from single-cell organisms to plants, animals and humans. Viruses have their own helicases encoded in their genomes as well. It also appears that they all have a common ancestor. In every eubacterial or eukaryotic organism that has been sequenced until now, the RecQ genes have been identified. (Killoran & Keck, 2006) Even though they were characterized and their sequences and motifs are known, their functions and working mechanisms are not yet fully understood. In 1993, Gorbalenya and Koonin proposed a classification system for helicases, which was sequence-based. It contained three superfamilies (SF), which were further divided into families, and two stand-alone families. This classification is still generally valid and can be used. As more helicases were found and helicase structures were examined, strong structural conservation was found within superfamilies and families, especially in SF1 and SF2. Differences were found between other helicases, however, which led to a modification of the helicase classification system, presented by Singleton et al. in 2007 in Annual Review of Biochemistry, keeping superfamilies 1 and 2 and reassigning the rest of helicases into four new superfamilies (3 –

6). Both DNA and RNA helicases are found in each superfamily, except for SF6 which only contains DNA helicases. (Jankowsky & Fairman-Williams, 2010)

All helicases have directional polarity for unwinding nucleic acids, either 3‘ to 5‘ or 5‘ to 3‘ with respect to the nucleic acid strand on which they translocate. (Patel & Domez, 2006) Two mechanisms of DNA double helix unwinding by DNA helicases were proposed – passive and active. Passive mechanism proposes that helicase itself does not unwind, only moves forward and catches released ssDNA separated by thermal fluctuation. Active mechanism on the other hand would actively destabilize dsDNA ahead of fork and use second DNA-binding domain to trap and hold unwound ssDNA. (Sharma, Doherty, & Brosh Jr., 2006)

2.1. RecQ helicases

RecQ helicases got their name after RecQ gene from Escherichia coli, the first discovered member of the family. They belong to the helicase superfamily II (SFII). Studies in both E.coli and human cells have suggested that although RecQ helicases act as recombination supressors most of the times, under certain circumstances the recombination can be, on contrary, promoted by them.

In yeast, usually single RecQ helicase is expressed. In mammalian cells, five different RecQ–family helicases were identified. In human cells, these are named RecQ1, BLM, WRN, RecQ4 and RecQ5. Deficiencies and mutations in their genes can lead to several genomic disorders, three of RecQ-family helicases are involved in autosomal recessive disorders that share common characteristics, like premature aging or cancer predisposition. (Mohaghegh & Hickson, 2001) Bloom syndrome is caused by mutations in the BLM gene, while Werner syndrome results from mutations in the WRN gene. Mutations in the RecQ4 gene can result in Rothmund – Thomson syndrome. However, germ line defects in the RacQ4 gene were also linked to RAPADILINO syndrome (Siitonen, Kopra, Kaariainen, & Haravuori et al., 2003) and Baller – Gerold syndrome. (Van Maldergem, Siitonen et al., 2006) Usual effects of RecQ4 gene mutations at the cellular level are aneuoploidy (change in the number of copies of a specific chromosome, in this case especially trisomy) and high frequency of chromosomal rearrangements, other signs of genomic instability can also appear. There are currently no known disorders linked to either RecQ1 or RecQ5.

The helicase activity was originally found in all but one RecQ helicase, RecQ4. Until recently, there had been no proof that RecQ4 actually works as helicase and unwinds double-stranded DNA, which raised the question whether RecQ4, unlike other RecQ-family helicases, possesses the unwinding function at all. In 2009 it was however proven that RecQ4 indeed is a functional DNA helicase. (Xu &

Liu, 2009) RecQ4 shows strong DNA re-annealing activity which probably masked its helicase activity in previous studies.

All RecQ proteins possess the helicase domain with conserved motifs which catalyze DNA unwinding. This domain remained highly conserved in evolution from bacteria to human cells. (Sharma, Doherty, & Brosh Jr., 2006) The RecQ helicase domain contains seven motifs common for all SFI and SFII helicases – motifs I, Ia, II, III, IV, V and VI. Motifs I and II have a significant role in ATP binding and hydrolysis, they are also known as Walker A and Walker B sites. (Bennett & Keck, 2004) There is also RecQ-specific motif 0, N-terminal to Motif I, found both in bacterial and eukaryotic proteins. It shows similarity to Q motif of DEAD-box RNA helicases and is supposed to have ATP binding function. (Bernstein & Keck, 2003) Motif Ia contributes to ssDNA binding, while motif III plays an important role in unwinding. Defects in nucleic acid binding in motif IV-mutants suggest that motif IV arranges conformational changes and therefore is required by helicase for moving along the substrate. (Tuteja & Tuteja, 2004) Interestingly, in contrast to other RecQ helicases, RecQ4 helicase does not have C-terminal RQC motif (containing Zinc-binding domain, which was suggested to participate in DNA binding and protein folding (Liu, Rigolet, Dou, Wang, & Xi, 2004)). RecQ4 also does not contain HRDC motif (which probably helps recognize the DNA as the substrate (Bernstein & Keck, 2003)), found in many DNA-metabolizing proteins including most members of RecQ family, WRN and BLM in human cells, for example. On the other hand, first 200 N-terminal amino acids of RecQ4 show homology with the yeast replication initiation factor Sld2, which implicates that RecQ4 participates on replication initiation in human cells as well. (Sangrithi, et al., 2005) RecQ4 can also interact with the ubiquitin ligases, UBR1 and UBR2, though the function of these complexes is currently unknown. (Hanada & Hickson, 2007) Some of the RecQ proteins also contain strongly acidic regions, usually located N-terminal to helicase domain. Experiments suggest that these regions may be important for binding replication protein A (RPA). These are most prominent in WRN. (Doherty, et al., 2005) WRN protein is also unique among RecQ helicase as it carries N-terminal exonuclease domain. (Bennett & Keck, 2004) RecQ5 can exist, unlike other RecQ helicases, in several different isomers, depending on

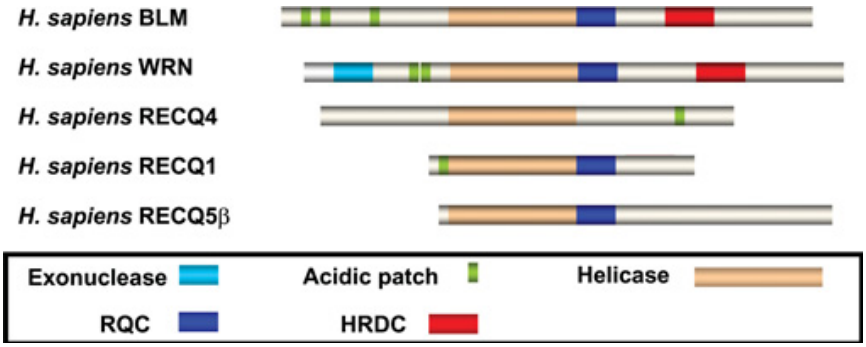

Fig. 1 Schematic representation of the conserved domains and motifs in human RecQ-family helicases (RecQ5α and RecQ5γ not included). (Sharma, Doherty, & Brosh Jr., 2006)

RecQ5 gene transcript splicing. Isomers RecQ5α and RecQ5γ are small and localize into cytoplasm, while RecQ5β isomer is significantly bigger and localizes into nucleoplasm (like other human RecQ helicases). (Shimamoto, Nishikawa, Kitao, & Furuichi, 2000) This large isomer also contains the long C-terminal region, which posesses strand-annealing activity. (Garcia, Liu, Jiricny, West, & Janscak, 2004)

Results of several experiments suggest that RecQ4 expression is tissue-specific. Originally it was assumed the expression would be particularly significant in the thymus and testis. (Kitao, Ohsugi, Ichikawa, Goto, Furuichi, & Shimamoto, 1998) These results were not confirmed, instead the chodrocytes of developing bones and cartilages and the proliferating intestinal enterocytes of embryonic mice were marked as the places of more prominent RecQ4 expression. (Siitonen, Kopra, Kaariainen, & Haravuori, 2003) More recent results point especially at embryonic and early postnatal development stages, possibly linking the phenotypic manifestations of RecQ4 deficiency to these expression patterns (for example bone dysplasia or intestinal villus atrophy). (Kellermayer, 2006) BLM was also shown to be expressed in specific tissues, mainly in lymphoid tissues and in skin and digestive tract, mostly in proliferating cells. (Turley, Wu, Canamero, Gatter, & Hickson, 2001)

3. Diseases connected to RecQ helicases

Unlike Bloom's syndrome and Werner's syndrome, which are both specific diseases caused by mutations in specific helicase genes (BLM and WRN, respectively), mutation in RecQ4 can result in one of three different disorders: Rothmund-Thomson syndrome, RAPADILINO syndrome and Baller-Gerold syndrome, which was linked to RecQ4 more recently. Even though a range of different mutations have been identified for each syndrome (incluing intronic deletions, frameshifts, splice site mutations, missense and nonsense mutations), there is still no explanation of the fact that these mutations can result in three distinct disorders (even though they share many common symptoms). (Hanada & Hickson, 2007) Recently, single nucleotide polymorphism in RecQ1 was suggested to be causing significantly decreased survivability of pancreatic adenocarcinoma. (Donghui, et al., 2006)

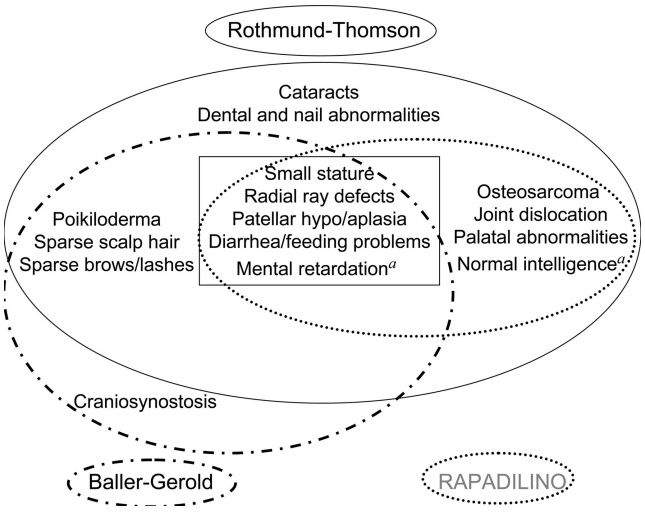

Fig. 2 The list of clinical symptomes of RecQ4 related syndromes, divided by specificity to each disease. Mental Retardation was only occasionally noted in RTS and RAPADILINO cases. (Kellermayer, 2006)

3.1. Rothmund-Thomson syndrome

Rothmund-Thomson syndrome (also RTS) is one of three diseases caused by mutation in RECQ4 gene. Originally, this syndrome was described as two separate medical conditions by Auguste Rothmund and Sydney Thomson and were later connected as two parts of the same entity by William Taylor. (Kellermayer, 2006) RTS is an autosomal recessive disorder, and its main characteristics are poikiloderma (the medical condition when the patient has areas of skin with increased or decreased pigmentation), sparse hair and eyebrows, cataracts, growth retardation, gastrointestinal problems, skeletal and dental abnormalities and joint dislocations and predisposition to cancer (especially osteosarcoma – bone cancer). This disorder starts developing in early stages of age. First symptoms (red patches on skin) show up between the age of three and six months, subsequently developing into poikiloderma and other symptoms. RTS has been linked in a subset of cases to RecQ4 helicase in 1999 by Kitao et al. Interestingly, not all patients suffering from RTS carry mutated RECQ4 gene – the genetic changes were only found in approximately 60% of cases, though it was determined that all patients that developed osteosarcoma (but did not suffer from cataracts) carried at least one deleterious mutation in RECQ4 gene. This discovery led to defining two types of RTS – Type I which causes poikiloderma and cataracts, but not osteosarcoma (this form was described by A. Rothmund), and Type II which is associated with deleterious mutations in RECQ4 gene and leads to osteosarcoma. These two Types were also described on mouse mutants. (Mann, Hodges, Barnes, Vogel, Hassold, & Luo, 2005)

Fig. 3 RTS patient (from Atlas of Genetics and Cytogenetics in Oncology and Haematology, http://atlasgeneticsoncology.org/Kprones/Images/RothmundID10021_1.jpg)

3.2. RAPADILINO syndrome

RAPADILINO syndrome is also an autosomal recessive disorder which is characterized by several symptoms, which also create its name – radial hypoplasia or aplasia (RA), patellar hypoplasia or aplasia and cleft or high arched palate (PA), diarrhoea and dislocated joints (DI), little size and limb malformation (LI), and nose slender and normal intelligence (NO). When comparing them to RTS, the

most obvious difference is the lack of ectodermal symptoms, such as poikiloderma or sparse hair. Also, RAPADILINO patients have lower cancer prevalence. This could be explained by the fact that unlike RTS patients, RAPADILINO patients mostly have the helicase domain intact. Most of the observed cases of this disease occured in Finland, only few cases appeared in other countries. (Hanada & Hickson, 2007) All patients from Finland also shared the same splice site mutation in RecQ4, IVS7+2delT in intron 7. Both homozygotes and heterozygotes for this mutation were found amongst patients. (Siitonen, Kopra, Kaariainen, & Haravuori, 2003)

3.3. Baller-Gerold syndrome

Third identified autosomal recessive disorder connected to RecQ4 is recently identified Baller-Gerold syndrome (also BGS). Again, this disease shares many common symptoms with previous RecQ4-connected syndromes, such as radial ray hypoplasia, skeletal dysplasia or short stature. Also, there is significant clinical overlap with other, non-RecQ4-related syndromes, such as Fanconi anaemia or TWIST mutations. Due to this fact, it is sometimes necessary to perform additional steps (such as chromosomal analysis, TWIST gene sequencing) to correctly identify BGS. It can also be mistaken with RTS in early age. (Van Maldergem, Siitonen, & al., 2006) BGS-specific symptom is craniosynostosis (changed skull growing pattern during infancy, resulting in different skull shape; caused by premature ossification of fibrous sutures). Craniosynostosis has not been observed in RTS or RAPADILINO cases so far. Also, BGS patients were not reported to show predisposition to cancer yet. (Hanada & Hickson, 2007)

Fig. 4 BGS patient (Van Maldergem, Siitonen, & al., 2006)

Fig. 5 Bloom syndrome patient (from Massachusetts Institute of Technology, http://web.mit.edu/biology/guarente/human/human.html)

3.4. Bloom syndrome

Bloom syndrome (BS) is an autosomal recessive disease caused by mutation in BLM helicase gene. Typical symptom is severe growth retardation. Remarkably, BS cells usually appear normal in size, which indicates that the newborn has an abnormally small amount of cells. Also, BS strongly increases the probability of cancer development. (German J. , 1993) Abnormal skin pigmentation and facial skin rash induced by direct sunlight are also a typical symptoms. Some BS patients also display mental

retardation, immunodeficiency, diabetes or mild anaemia. Tumours of various types tend to develop in earlier stages of life in affected individuals (with exception of melanoma, possibly due to low exposure to sunlight), even quite rare types like osteosarcoma or medulloblastoma. Also, the cases with multiple independent tumours appeared. (Hanada & Hickson, 2007) Men affected by BS are infertile and cannot produce mature sperm. Women, on the other hand, are usually fertile and several pregnancies have already been noted. (German J. , 1993) Interestingly, an elevated frequency of BLM mutation has been found in the Ashkenazi Jewish population. (German, Sanz, Ciocci, Ye, & Ellis, 2007)

3.5. Werner syndrome

Werner syndrome (WS), yet another autosomal recessive disorder, is caused by mutation in WRN helicase gene. The symptoms of this disease could be described as „progeroid" – it is typical for patients to show the symptoms of premature aging. These symptoms usually start displaying themselves during puberty and include greying and subsequent loss of hair, skin and voice changes, shortness of stature, diabetes and cataracts. The average age of death is 47. (Epstein, Martin, Schultz, & Motulsky, 1966) Similarly to BS, affected men are infertile, whereas this is not generally true for women (pregnancies were recorded as well). Premature aging could be connected to the fact that WS cells show decreased ability to proliferate. It is worth noting that majority of documented patients are of Japanese origin. (Hanada & Hickson, 2007)

Fig. 6 Werner syndrome patient (from International Registry of Werner Syndrome, University of Washington, http://www.pathology.washington.edu/research/werner/registry/registry.html)

4. Cell cycle

Every living organism tries to preserve itself and to pass its genetic information on as many descendants as possible (given the limits of the organism itself and the environment it's habitating). For any action connected to these goals, the cell division is essential. Single-cell organisms divide into new organisms, in multicellular organisms long and complex sets of divisions occur in order to replace dead cells or to participate in the growth of the offspring. The details of cell divison differ more or less in each case, but the basic sequence of events is universal and is generally called the cell cycle.

The cell cycle is generally divided into several phases, each one being marked by a different cell processes. (Hartwell & Weinert, 1989) The first major phase is the S phase („synthesis"). During this phase, the DNA is duplicated. S phase takes usually around 10 – 12 hours in mammalian cells and can

account for up to half of the cell cycle. The second major phase is the M phase („mitosis“), a phase during which the cell is divided. This phase is significantly shorter compared to S phase. It usually lasts less than an hour in a mammalian cell. More details on mitosis are described in the next chapter. Between these major phases there are usually G phases („gap“), which serve mainly as the resting periods in which the cell has the opportunity to grow and gather material and energy for the next step of the cell cycle. The G phases are G1 that comes after mitosis and G2 that takes place after S phase. The G1, S and G2 phases can also be designated together as the interphase.

If the phases came in rapid succession, the newborn cells would be smaller with every generation and would be lacking building components to continue. In order to prevent that, there are many cellular control mechanisms which prevent the cell from progressing to next phase unless given prerequisites are met. (Hartwell & Weinert, 1989) These control mechanisms make second important function of G phases, and if the conditions for continuing are not met, the cell can idle in either G phase or even slip into a resting state called G0 phase. In this phase, the cell can remain for very long time without proliferating, waiting for more favorable conditions to resume progression of the cell cycle. Many cells in multicellular organisms, for example hepatocytes in mammalian bodies, are to stay permanently in G0 phase, or even withdraw from the cell cycle completely (the nerve tissue, for example), never proliferating again. (Hall & Watt, 1989)

4.1. Mitosis

The M phase consists in fact from two main events – the nuclear division (mitosis) and the cytoplasmic division (cytokinesis). The initiation of M phase is triggered by CDK1 (more on cyclin-dependent kinases in the chapter Cell cycle control), inducing a cascade of protein phosporylation events. Subsequently, the chromosomes condense, the nuclear membrane is dismantled and partially fuses with the endoplasmic reticulum (ER), which undergoes significant reorganization. (Puhka, Vihinen, Joensuu, & Jokitalo, 2007) Also the cell adhesion to the extracellular matrix is decreased. It is very important for the cell to ensure that mitosis and cytokinesis take place in correct order – dividing into daughter cells before chromatide segregation is completed would be disastrous. Because of that, there are at least two mechanisms to prevent such thing from happening. First, cytokinesis cannot occur until some of the mitosis-related proteins are deactivated, CDK for example. (Howell & Lew, 2012) Second, the cell cannot divide until the spindle finished segregation and formed a central spindle, which is required to maintain the contractile ring functionality. It should be noted, however, that he beginning of cytokinesis overlaps with the end of mitosis, starting during anaphase.

Before the M phase itself can be initiated, replication of the DNA must be completed. Also, in animal cells, the centrosome duplication must occur. That way it is ensured that each daughter cell inherits the complete copy of the genome and its own centrosome. The duplicate centrosomes are important for proper spindle formation and function, too.

At the beginning of M phase, chromosome duplicates are closely connected by protein complexes called cohesins. These bonds are strong and are created between sister chromatids during DNA replication. (Gandhi, Gillespie, & Hirano, 2006) The chromosomes also start to condense into short and highly compact form, that is clearly visible under microscope. This process is started by above-mentioned CDK1, which phosporylates proteins called condensins. Condensins can hydrolyze ATP and use the energy to create DNA coils. They also work together with cohesins, the result is therefore a complex of two condensed sister chromatids organized along the central axis.

Mitosis is divided into five main stages; in plant cells, there is a sixth recognized phase – preprophase, which precedes prophase and whose purpose is to determine proper division planes prior to starting mitosis itself. (Ambrose & Cyr, 2008) All phases are happening in given sequence, which is the same for all cells, from bacteria to humans. First of all mitosis phases is the prophase. During prophase the chromosome condensation occurs, the centrosomes have moved apart and started forming mitotic spindle between them. When CDK1 phosporylates directly the nuclear lamina, nuclear membrane breakdown is triggered (Güttinger, Laurell, & Kutay, 2009) and the second phase of mitosis – prometaphase – is started. The disassembly of nuclear membrane allows direct access to condensed chromosomes to microtubules growing from centrosomes. They start attaching to kinetochores which are located in the centers of chromosomes, locations called centromeres. Attached tubulins stop degradating and stabilize. Each chromosome is connected to both centrosomes, eventually forming a mitotic spindle, the complex structure whose purpose is to ensure proper separation of genome. (Karsenti & Vernos, 2001) When all chromosomes are attached, third phase – metaphase -

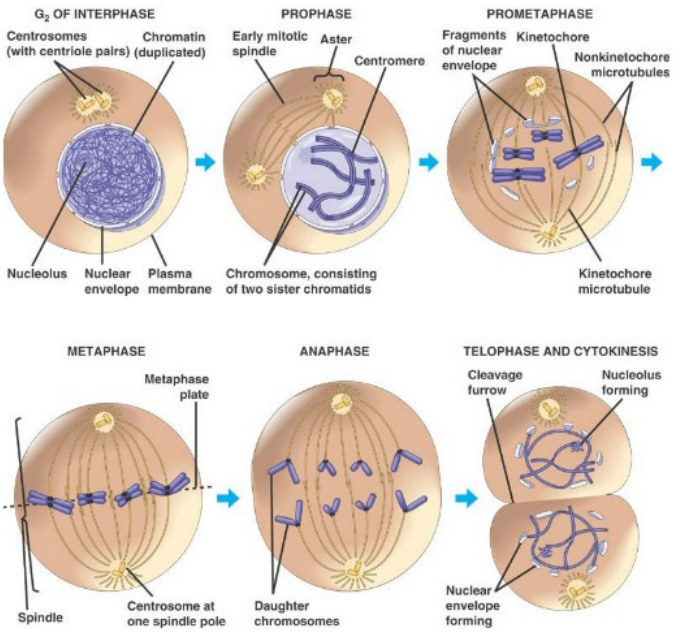

Fig. 7 Mitosis phases (from Lyon Institute of Technology, http://www.nanoiv.com/index.php?pub=applic_nano&art=cellimaging)

begins. During this phase, the chromosomes are pushed and pulled by microtubules, eventually positioning in between the centrosomes, forming so-called metaphase plate.

At this point, an important cell cycle checkpoint is located, the mitotic spindle checkpoint. Next mitotic phase, anaphase, cannot begin unless all chromosomes are properly attached and aligned in the spindle. It is assumed that improperly attached kinetochore transmits a signal that prevents progression. (Musacchio & Salmon, 2007) At this point, the anaphase-promoting complex (APC) is activated, triggering the metaphase-to-anaphase transition. APC is essentially an E3 ubiquitin ligase, capable of tagging several important proteins for degradation. Most prominent among its targets are securin and mitotic cyclins. The degradation of mitotic cyclins leads to inactivation of CDK1. The degradation of securin leads to activation of protease called separase and subsequent cleavage of cohesins binding the sister chromatids. Chromatids are then able to be pulled away to opposite poles. (Peters, 2006) The sister chromatid separation is achieved through two different processes. First one, called anaphase A, is the initial movement of chromosomes toward poles, generated mainly by shortening of kinetochore microtubules, although there is also some depolymerization of spindle microtubules at the spindle poles happening. The second process, referred to as anaphase B, means the separation of the poles themselves. (Rath & Sharp, 2011) It is important to note that both anaphase A and anaphase B processes are occuring at the same time. At the end of anaphase, sister chromatids are separated at opposite poles and are beginning to decondense. At the end of this phase, the remnants of nuclear envelope start reforming around each group of chromosomes. The last phase of mitosis is called telophase. Here, nuclear envelope is reassembled around each set of chromosomes, nuclear lamina reforms, nuclear pore complexes are incorporated. (Güttinger, Laurell, & Kutay, 2009) When the reformation of nuclear envelope is completed, chromosomes fully decondense and gene transcription ability is resumed. With that, mitosis is ended and cytokinesis can be concluded (as mentioned above, the cytokinesis has already started during anaphase).

4.2. Cell cycle control

As mentioned above, there are many control mechanisms included in cell cycle, preventing the cell from starting demanding actions (such as DNA replication) in unfavourable conditions. Also, sometimes one step of the cycle takes longer than expected, for example due to the lack of resources, in which case the cycle needs to be slowed down. That's why there are usually several specific places in the cell cycle in which the cycle can be stopped (also „arrested"). These places are called checkpoints. (Hartwell & Weinert, 1989) Most significant checkpoints are G1 and G2 checkpoint, located at the end of G1 phase and G2 phase respectively. Their purpose is to make sure that the environment is stable and favourable for the cell and, in case of G2 checkpoint, if all DNA has

been replicated during S phase. Another important checkpoint is located in M phase, so-called mitotic spindle checkpoint, which controls that all chromosomes are attached to the mitotic spindle before progressing to anaphase. (Musacchio & Salmon, 2007) Last but not least, S phase checkpoints are protecting the dividing cell from DNA damage. (Bartek, Lukas, & Lukas, 2004)

The most important family of proteins participating in cell cycle control are cyclin-dependent kinases (CDKs). There are several different types of these proteins and their activity depends on the progress of the cell through the cycle. As the name suggests, CDKs cannot function without regulating proteins called cyclins. These proteins are being synthesised and degraded throughout the cell cycle. When the cyclin binds to associated CDKs, they form the cyclin-CDK complex. Such a complex can usually phosporylate large amount of different proteins, directly participating in all major events of cell cycle, including DNA replication, mitosis and cytokinesis. When it becomes necessary to lower the amount of active CDKs, for example at the end of mitotic phase, the cell can employ several different means to achieve that, such as cyclin degradation or cyclin-dependent kinase (Visintin, Craig, Hwang, Prinz, Tyers, & Amon, 1998)There are four major classes of cyclins in mammalian cells: D-, E-, A- and B-type cyclins. B-type cyclins seem to reside mainly in cytoplasm, other classes are usually found in nucleus. It is worth noting that whereas yeast only expresses single major cyclin-dependent kinase, CDK1, participating in all phases of cell cycle, in higher organisms there are approximately 20 more specialized phase-specific CDKs (Satyanarayana & Kaldis, 2009)

5. Function of RecQ helicases during cell cycle

Since the helicases were discovered, an important question arose: what is their exact cellular function? As of today, it is still not fully answered, but we now have at least some idea. Different RecQ helicases were researched to different extent, therefore the function of some of them is more clear to us than the rest. Generally, RecQ helicases participate in replication pathways, in protecting the cell from excessive recombination, in unwinding secondary DNA structures that may block the replication fork and last but not least in telomere maintenance. (Bennett & Keck, 2004)

5.1. BLM

BLM helicase is probably the most explored helicase from the RecQ family and some of its functions were reliably determined. BLM was shown to interact with DNA topoisomerase IIIα (Topo IIIα). It mediates a contact of the topoisomerase with DNA and can stimulate this topoisomerase to function on negatively supercoiled DNA molecules. Moreover, BLM in conjuction with Topo IIIα can catalyze dissolution of double-Holiday junctions that are formed during homologous recombination. (Wu & Hickson, 2003) BLM was also shown to be able to recruit Topo IIIα to single-stranded DNA structures.

(Wu & Hickson, 2002) Another important protein BLM interacts with is hRMI1 (also BLAP75), which is important for BLM-TopoIIIα complex stability (Yin, et al., 2005) and can significantly increase the rate of double Holliday junction dissolution. (Wu, et al., 2006) More recently, another interacting protein was found – RMI2 (also BLAP18). This protein helps stabilize the protein complex of BLM, TopoIIIα and hRMI1 and plays an important role in correct targeting of this complex. (Singh, et al., 2008)

BLM is, like other RecQ helicases, able to unwind several DNA structures, including G-quadruplex (G4) DNA, which can lead to replication fork stalling and collapse (Karow, Wu, & Hickson, 2000) and can cause the double-strand breaks. The cells of patients with Bloom's syndrome show an elevated level of recombination events, suggesting that BLM can regulate and suppress these events, having for example the capability of unwinding Holliday junctions. (Karow, Wu, & Hickson, 2000) BLM in complex with TopoIIIα was also confirmed to be able to solve the double Holliday junction without the exchange of genetic material between the two involved DNA molecules. The loss of this activity in cell could explain several cell phenotypes connected to Bloom syndrome. (Wu & Hickson, 2003)

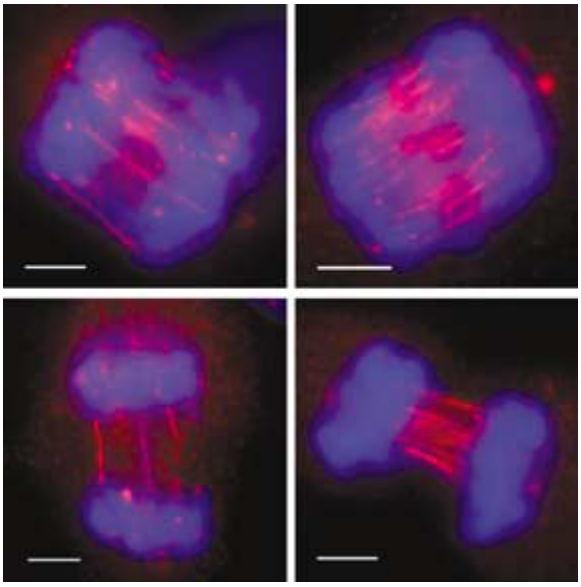

Fig. 8 Anaphase cells with visible BLM-DNA bridges after being treated with 10 or 50 µM ICRF-159. Scale bar – 5 µm. (Chan, North, & Hickson, 2007)

Another very important function of BLM helicase was defined fairly recently – the ability to detect the anaphase bridges, very thin DNA structures connecting sister chromatids, and the ultrafine bridge-like threads, which connect the segregating daughter nuclei and cannot be detected using conventional DNA dyes, and bind to it. Since BLM-deficient cells show significantly higher frequency of these bridges, it was suggested that BLM helicase has an important role in resolving them (in complex with Topo IIIα and RMI1). (Chan, North, & Hickson, 2007) Another protein was shown to localize to ultrafine threads – PICH (Plk1-interacting checkpoint helicase), a SNF2 family helicase which was found as an interaction partner of the mitotic kinase Plk1. (Baumann, Körner, Hofmann, & Nigg, 2007) PICH localizes to kinetochores during prometaphase, in anaphase then moves to ultrafine threads, binds to BLM and mediates BLM localization to ultrafine threads. In cells with either PICH or BLM depleted, however, these threads contained histones. This suggests that PICH-BLM complex serves as a limiting factor for histone binding on ultrafine anaphase threads, which allows these threads to stretch to larger distances without breakage, preventing the formation of nucleosomes on them and therefore maintaining the

centromere integrity during mitosis. (Ke, et al., 2011) The inability to resolve anaphase bridges correctly may lead to aneuploidy (Chan, North, & Hickson, 2007). Depletion of either PICH or BLM also causes significantly higher frequency of micronuclei formation during telophase (up to 30% cells). These micronuclei often contain centromere markers, as more than half of ultrafine threads connect centromeres. PICH depletion however does not change the rate of sister chromatid exchanges, suggesting that PICH has no influence on BLM's repair functions. (Ke, et al., 2011)

5.2. RecQ1

Of all RecQ helicases, RecQ1 is the most abundant in human cells (Wu & Brosh Jr, 2010), yet its is one of the least characterized RecQ helicases. RecQ1 homologues were found in number of organisms, including *Neurospora crassa* (Kato, Akamatsu, Sakuraba, & Inoue, 2004) or some plants, for example *Oryza sativa*. (Saotome, Kimura, Mori, Uchiyama, Morohashi, & Sakaguchi, 2006) Although there is no disease genetically linked to this protein yet, studies suggest that RecQ1 plays an important role in DNA damage response and maintaining genome integrity. (Wu & Brosh Jr, 2010) In response to replicational stress or DNA damage, RecQ1 undergoes relocalization from nucleolus to the nuclear compartment (specifically to chromatin) and is phosphorylated. RecQ1 also may have a regulatory role in cell proliferation, as RecQ1-depleted cells show impaired ability to synthesize DNA. RecQ1-depleted human cells have reduced ability to respond to the effects of ionizing radiation. The frequency of sister chromatid exchange in these cells is also higher compared to normal cells, which indicates the accumulation of DNA double-strand breaks. Research results show that following the exposure to ionizing radiation, RecQ1-depleted cells display defective maintenance of G2/M checkpoint. (Sharma & Brosh Jr, 2007)

5.3. RecQ5

RecQ5 helicase was shown to share some functions with BLM, such as lowering the frequency of sister chromatid exchanges. Their operational pathways are not the same, however, as the frequency of crossovers rises even more when both BLM and RecQ5 are depleted from the cell. (Hu, Lu, Barnes, Yan, Lou, & Luo, 2005) Study showed that RecQ5β isomer is capable of binding to topoisomerases IIIα and IIIβ, which suggested that this helicase might be an active DNA helicase participating in DNA metabolism in nucleoplasm. (Shimamoto, Nishikawa, Kitao, & Furuichi, 2000) Helicase activity was indeed later proved, also the single-strand annealing activity was shown. The strand-annealing activity of RecQ5β was however inhibited when RPA was present. (Garcia, Liu, Jiricny, West, & Janscak, 2004)

Further studies shown that RecQ5β is capable of promoting strand exchange in a synthetic fork structure mimicking stalled replication fork, although only being able to actively unwind the lagging strand. The helicase was also shown to localize to the sites of stalled replication forks *in vivo*, suggesting the function of bypassing DNA damage by template switching. (Kanagaraj, Saydam, Garcia, Zheng, & Janscak, 2006) Zheng et al. (2009) also proved that RecQ5 colocalizes with MRN complex (MRE11-RAD50-NBS1) in response to replication fork arrest or chromosomal breakage and that in fact RecQ5 cannot be recruited to these sites without the presence of MRN complex. (Zheng, et al., 2009) RecQ5 helicase was also found to interact with topoisomerase IIα, mostly during S-phase of the cell cycle. As a result, the helicase activity of the helicase is suppressed and the decatenation activity of the topoisomerase is enhanced. During mitosis, when the RecQ5 was depleted, the number of undercondensed or entangled chromosomes rose rapidly during metaphase. Overall, the survivability of the culture decreased, with lot of cells stopping at G2/M checkpoint and undergoing apoptosis. Results of this study suggested that RecQ5-TopoIIα complex is important for enhancing the stability of eukaryotic chromosomes. (Ramamoorthy, et al., 2012)

Recent studies on *Drosophila melanogaster* RecQ5-deficient syncytial embryos also showed that in the absence of RecQ5, the number of DNA (probably histone-containing – stainable with DAPI) anaphase bridges significantly increases, which may lead to double-strand breaks in daughter nuclei or incorrect connection of segregating chromosomes during anaphase or telophase. This in turn causes the cell to exit the cell cycle prematurely. These observations suggest that RecQ5 plays a significant role in anaphase bridge resolving and/or prevention. (Sakurai, Okado, Ito, & Kawasaki, 2011) Rad51 filaments which bind to ssDNA and increase the number of homologous recombination events are also disrupted by RecQ5, in an ATP-dependent manner. In order to function properly, RecQ5 forms a complex with Rad51, however the dissociation continues to certain extent even with RecQ5 mutants incapable of interacting with Rad51. Binding of Rad51 to ssDNA is a critical step of homologous recombination and this interaction might be a significant part of RecQ5 anti-recombinase function. (Schwendener, et al., 2010) The results on mice models also gave rise to speculations that RecQ5 gene might be a cause for a cancer-prone syndrome. (Hu, Lu, Barnes, Yan, Lou, & Luo, 2005)

5.4. WRN

WRN is not participating so distinctively in mitosis as other RecQ helicases, it is nevertheless important for the successful conclusion of the cell cycle due to its role in DNA damage repair and telomere maintenance. This helicase stands out between the RecQ helicases, because it possesses the 3‘-5‘ exonuclease activity. This activity is activated on substrates with alternative structures

(bubble, single-strand loop) or containing Holliday junction and used, in cooperation with the helicase unwinding activity, to solve these structures. (Shen & Loeb, 2000) In additon to participating in these DNA damage repair pathways, WRN is also suggested to help solving double-strand breaks. (Opresko, Cheng, von Kobbe, Harrigan, & Bohr, 2003)

WRN participates in many cellular processes, including replication initiation (WRN is capable of interacting with several proteins critical for DNA replication (Opresko, Cheng, von Kobbe, Harrigan, & Bohr, 2003), also WS patients' cells show decreased frequency of replication initiation (Takeuchi, et al., 1982)), recombination or DNA damage repair, helping to eliminate alkylated or oxidative DNA damage. (Harrigan, et al., 2003) WRN is able to interacts with many cellular proteins, with many different effects (not all of which are known at the moment). (Opresko, Cheng, von Kobbe, Harrigan, & Bohr, 2003) Worth noticing is the fact that WRN was proved to directly interact with BLM helicase *in vitro*, blocking WRN's exonuclease function and suggesting similar interaction can happen *in vivo*. However, exact function of this protein complex was not yet discovered. (von Kobbe, et al., 2002) Both RecQ helicases seem to share some similar, partially overlapping functions as well.

Interactions between WRN and Ku complex, as well as deletions at nonhomologous joining ends' sites in cells obtained from Werner syndrome patients, suggest that WRN participates in nonhomologous end joining, possibly preventing the loss of too many nucleotides during double strand break repair and thus preventing the creation of large and potentially dangerous deletions. (Oshima, Huang, Pae, Campisi, & Schiestl, 2002) WS cells were also shown to undergo significant reduction of successully concluded mitotic recombinations, suggesting that WRN plays a significant role in the mitotic recombination resolution. The cells which lost WRN fuction showed increased probability of cell cycle arrest or cell death. (Prince, Emond, & Monnat Jr, 2001) Newer study of Rahn et al. (2010) came with slightly different results, suggesting that WRN in fact serves as a suppressor of mitotic homologous recombination events in the cells with DNA damage, preventing the genomic instability resulting from excessive and inappropriate homologous recombiantion. (Rahn, Lowery, Della-Coletta, Adair, & Nairn, 2010)

It was shown that WRN-deficient cells fail to properly activate their decatenation checkpoint, which can subsequently lead to increased amount of chromosomal damage and the induction of apoptosis. (Franchitto, Oshima, & Pichierri, 2003) It was also suggested that this helicase plays an important role in the replication of telomeres and that the removal of the helicase leads to loss of telomeres on sister chromatids. (Crabbe, Verdun, Haggblom, & Karlseder, 2004) This effect could be the cause of elevated cancer occurence in patients with Werner syndrome. (Crabbe, Jauch, Naeger, Holtgreve-Grez, & Karlseder, 2007)

5.5. RecQ4

RecQ4 is not so well understood as BLM or WRN, despite being one of known disease-causing RecQ helicases, and its functions are yet to be fully determined. Recent studies however shed some light on this problematics. RecQ4 was shown to have the ssDNA-annealing activity, inhibited by presence of RPA (Macris, Krejci, Bussen, Shimamoto, & Sung, 2006) and, as mentioned above, Xu & Liu (2009) also proved it possesses unwinding activity as well. (Xu & Liu, 2009) Study on RecQ4–deficient fibroblasts found that such cells are highly sensitive to some chemical agents (hydroxyurea, for example) and also show modest sensitivity to UV light and ionizing radiation. (Jin, Liu, Zhang, Otta, Plon, & Wang, 2008) It was further discovered that RecQ4 accumulates at sites of laser-induced DNA damage and that RecQ4-deficient cells show higher frequency of double-strand breaks. (Singh, et al., 2010) In response to oxidative stress, RecQ4 was shown to accumulate in nucleoli, suggesting that it plays a role in the response to such danger. (Woo, Futami, Shimamoto, Furuichi, & Frank, 2006)

Mann et al. (2005) has generated a mouse model with RecQ4-deficient cells. Significant percentage of mice embryo fibroblasts (compared to wild-type cells), up to 24%, showed aneuploidy. Further analysis of different cell types also showed increased amount of aneuploidy compared to wild-type cells. To find the cause of higher aneuploidy rates, the cells were screened for chromosome instability during mitosis, in metaphase. Multiple cases of premature centromere separation was observed, which lead to sister chromatids being separated too soon and the consequent problems during chromosome segregation, which in turn can lead to aneuploidy. Interestingly, RecQ4-deficient cells possessed normal frequency of sister chromatid exchange and therefore probably does not participate significantly on mitotic recombination regulation, unlike BLM or WRN. (Mann, Hodges, Barnes, Vogel, Hassold, & Luo, 2005) Another study has suggested that RecQ4 plays an important role in replication fork arrest, since RecQ4-depleted cells showed defects in S-phase arrest when treated with hydroxyurea or irradiated with UV light. (Park, Lee, Beck, & Lee, 2006)

RecQ4 does not have the wide scale of interacting proteins like several other RecQ helicases (WRN, especially). However, it was proved to associate with replication origins and interact with MCM protein complex, which is important for DNA replication. The only similar interaction among RecQ helicases was proved by RecQ1 (Thangavel, et al., 2010), suggesting the unique role of RecQ4 in replication. (Xu, Rochette, Feyissa, Su, & Liu, 2009) Moreover, the depletion of RecQ4 from the cell caused sixfold reduction in proliferation, suggesting that RecQ4 is an important part of DNA replication system. (Thangavel, et al., 2010)

The C-terminal region of RecQ4 can interact with poly(ADP-ribose)polymerase-1 (PARP-1), nuclear protein participating in DNA repair, transcription and replication, further supporting the assumption

that RecQ4 helicase is involved in oxidative damage response mechanisms. (Woo, Futami, Shimamoto, Furuichi, & Frank, 2006) It was also shown to form stable complexes with ubiquitin ligases UBR1 and UBR2, which however didn't result in ubiquitylation or degradation. (Yin, Kwon, Varshavsky, & Wang, 2004) Rad51 is another interaction partner for RecQ4, colocalizing with it to sites of double-strand breaks and adding another fact supporting the theory that this RecQ helicase helps prevent DNA damage caused by double-strand breaks. (Petkovic, Dietschy, Freire, Jiao, & Stagljar, 2005)

6. Conclusion

The function of RecQ helicases is still clouded and unclear. Only in last few years we are starting to understand what their role in the cell might be. It seems that RecQ helicases have much bigger influence on the cell than it was anticipated, participating on essentially every important step of proliferation. BLM has been studied quite thoroughly and many functions, as well as interaction partners, have been identified, most notably the ability to resolve ultrafine anaphase bridges. WRN is, similarly, capable of interacting with a great number of cellular proteins and also was shown to possess exonuclease activity, which makes it unique among RecQ helicases. Not much is known about the exact functions of RecQ1, however it seems to have an important role in DNA damage response mechanisms. RecQ5 exists, in fact, in several isomeric forms, with the β-form being the most active. According to several studies, this helicase seems to help solve problems with stalled replication forks. Also, although there's no definitive proof yet, it is suggested that in can be the cause of cancer-prone syndrome. RecQ4, although being the cause of three different syndromes, is not studied as thoroughly as BLM or WRN and the scientists are still uncovering new facts, suggesting the diversity of cell actions this helicase is influencing.

The fact that defects in some of these helicases cause severe conditions should increase the interest in researching these proteins, as well as the fact that the common symptom is significantly increased predisposition to cancer. Discovering how can the mutation in single helicase protein increase the risk of cancer so much might lead to the new way of fighting this dangerous disease. Also the effect of premature aging in Werner syndrome suggests the possibility to shed some light on the aging process, which isn't fully understood either.

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